

## SHORT COMMUNICATION



# Novel frameshift mutation of the *NR0B1*(*DAX1*) in two tall adult brothers

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## Abstract

NR0B1 (nuclear receptor subfamily 0, group B, member 1) is a transcription factor encoded by *DAX1* (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1) responsible for the development and maintenance of the steroidogenic tissues. In humans the *DAX1* mutations cause congenital adrenal hypoplasia (AHC) and hypogonadotropic hypogonadism (HHG) in boys. Here we report two brothers who were assessed by endocrinologist at the age of 51 and 43 because of their serious osteoporosis. They had been substituted with prednisolone since the age of 4 and 9 years because of their primary adrenal insufficiency (PAI). Due to their late puberty caused by HHG at the age of 16 and 17 years their heights were – 3.1 and – 3.3 SD, but then they had a significant growth during their adulthood and reached the + 1.85 SD and + 3.78 SD respectively. During this period, they received glucocorticoid supplementation, but the treatment of their HHG was inadequate. At the age of 51 and 43 years insulin tolerance test (ITT) and gonadotropin releasing hormone (GnRH) test confirmed their PAI and HHG. Genetic test performed at this time revealed a novel, four nucleotides deletion (del.586-571c.GGGC or 572-575c.GGGC) of *DAX1* gene. The two brothers with AHC and HHG caused by a novel *DAX1* mutation, reached tall final heights, despite of the disadvantageous prednisolone treatment during their childhood. We assume that the long-term lack of the sexual hormone substitution was a significant reason of their above average height as well as their serious osteoporosis.

**Keywords** Novel *NR0B1*(*DAX1*) gene mutation, AHC · Adrenal hypoplasia congenita, HHG · Hypogonadotropic hypogonadism

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## Introduction

The *DAX1* is located on the short arm of X chromosome and is also known as NR0B1 (OMIM\*300473), encoding an orphan nuclear hormone receptor (NHR) [1].

DAX-1 is expressed in embryonic stem cells, steroidogenic tissues (gonads, adrenals), ventromedial hypothalamus as well as pituitary gland in developing and adult tissues [2, 3]. The exact mechanism of DAX1 in different transcriptional processes remains unclear, but it has shown that it can function as a repressor during the transcription or can have a role as an RNA shuttling protein [1, 4–9].

In case of the loss of *DAX1* function due to mutations develops an AHC and HHG in boys, although the phenotypic variation of severity and the age of manifestation are highly variable [10–12]. Even animal models indicate that DAX1 also plays a primary role in the development of testis and spermatogenesis [13].

In AHC patients the permanent zone of adrenal cortex is absent and abnormally large fetal adrenal cells persist, resulting in a structural and functional disorganization of the adrenal gland with consequent different severity of glucocorticoids, mineralocorticoids and androgens deficiencies [14, 15]. In patients with AHC the current treatment indicates maintenance of glucocorticoid and mineralocorticoid replacement and appropriate symptomatic treatment during adrenal crises [12, 16].

HHG is caused by combined and variable deficiency of hypothalamic failure in gonadotropin-releasing hormone (GnRH) producing and pituitary defect in responsiveness to GnRH, resulting in reduced gonadotropin production [17]. Treating HHG in affected boys requires gonadotropins and/or sexual hormone substitution during puberty and lifelong [12].

Here we report two brothers who had been substituted with glucocorticoids since their childhood due to their AHC and partially with sexual hormone since their late teenage because of their HHG. The final diagnosis was achieved by genetic testing demonstrating a novel, four nucleotides deletion of *DAX1*.

## Case Reports

### Patient 1

The 51-age patient was referred to the endocrinologist because of a serious osteoporosis (T-score lumbar:  $-2.3$  hip:  $-2.5$ ). At this point his height was 188 cm and he had a hypogonad phenotype with gynecomastia and hypoplastic testes (2 ml  $\times$  2 ml).

He was born at term from an uneventful pregnancy with 4400 gramm birthweight, from non-consanguineous healthy average height parents. However, during family history turned out that but one of his maternal uncles died in infancy.

After an unremarkable early childhood, the 4-year-old boy was hospitalized several times because of the repeating classical symptoms of hypadrenia (hyperpigmentation, failed to thrive, stomachache, vomiting). Based on the clinical findings and laboratory results the diagnosis of primary hypadrenia was established. The possibility of 21-hydroxylase deficiency was excluded, but he received antituberculous treatment. Karyotype revealed 46,XY male genotype. Per os prednisolone 2.5 mg/die and decosterone intramuscular injection (1  $\times$  5 mg/week) were introduced.

Due to bilateral cryptorchidism he was treated with intramuscular HCG (human choriogonadotropin) but because of the inefficacy of this treatment he underwent an orchidopexy at age 11 years.

His puberty was delayed and since the age of 23 years he received regular HCG treatment (3000 IU/2 months) and then the level of testosterone achieved the 9 nmol/l, testis and pubic hair were growing. Because of compliance problem from the age of 25 years until 51 years he remained untreated with testosterone replacement, but he has been receiving glucocorticoid supplementation (hydrocortisone 40 mg/day).

His growth curve was unusual (13 years 147 cm:  $-1.26$  SD, 16 years 152 cm:  $-3.1$  SD, 23 years 166 cm:  $-1.68$  SD) but at adult age (51 years) he reached 188 cm (1.85 SD) and his weight was 95 kg (BMI: 26.9 kg/m<sup>2</sup>).

Exactly at this age the diagnoses of PAI and HHG were confirmed by gonadotropin releasing hormone (GnRH) and the Insulin tolerance tests (ITT) (Table 1). These tests revealed a low level of the gonadotropic hormones, peak luteinizing hormone (LH): 0.3 IU/l and peak Follicle stimulating hormone (FSH): 1.6 IU/l, low cortisol (3.5 nmol/l), elevated ACTH (Adrenocorticotrophic hormone) ( $> 330$  pg/ml) and low growth hormone (GH) level (peak: 0.25 mIU/l). The brain magnetic resonance (MR) imaging showed normal brain anatomy and a lack of pituitary anomalies.

Molecular genetic examination revealed a novel, four nucleotides deletion of *DAX1* gene confirming the diagnosis of congenital adrenal hypoplasia.

From that time his treatment regimen includes hydrocortisone 30 mg/day (13 mg/m<sup>2</sup>/day hydrocortisone), 0.1 mg fludrocortisone/day, mesterolone 50 mg/day, alendronic acid 70 mg/week and vitamin D 3000 IU/day. Upon androgen substitution (testosterone undecanoate 1000 mg) he had a priapism and he refused this therapy.

**Table 1** Hormone concentrations in fasting and after endocrine stimulation (ITT and GnRH) tests in Patient 1 and Patient 2

	Patient 1	Patient 2	Reference range
Age	51 years	43 years	
Basal hormone levels			
IGFI	169.5	96	92–244 UG/l Male: 35 years: 80–240 ng/ml Male: 45 years: 70–210 ng/ml
Prolactin	171.8	371.8	54–380 mIU/l
TSH	2.8	2.7	0.35–4.94 mIU/l
fT4	17	10.5	9–19 pmol/l
Testosterone	< 0.28	0.33	5.8–28.1 nmol/l
Renin activity	103.9	124.1	0.2–5.7 ng/ml/h
Aldosterone	2.6	2.1	30–400 pg/ml
Insulin tolerance test			
Lowest glucose	2.2	2.0	< 2.2 mmol/l or half of the initial value
Peak ACTH	> 330	111.2	7.2–63.3 pg/ml
Peak GH	0.25	0.11	0–6 ng/ml
Peak cortisol	3.5	27.3	8–25 µg/dl
GnRH test			
Peak LH	0.3	0.3	1.7–8.6 IU/l
Peak FSH	1.6	1.4	1.5–12 IU/l
LH/FSH	0.19	0.21	> 1

*ACTH* Adrenocorticotrophic hormone, *FSH* Follicle stimulating hormone, *fT4* free thyroxine, *GnRH* Gonadotropin releasing Hormone, *GH* Growth hormone, *IGFI* Insulin-like growth factor, *LH* Luteinizing hormone, *TSH* Thyroid stimulating hormone

## Patient 2

He is a younger brother of the Patient 1 and he was also evaluated by the endocrinologist at the age of 43 years because of a serious osteoporosis (T-score, lumbar – 2.6, hip: – 2.5). His height was 200 cm presenting with a hypogonad phenotype: gynecomastia, 2 ml testes and a hypoplastic penis.

He was born from an uneventful pregnancy at term with 4700 gr birth weight. At the age of 9 years, based on the clinical symptoms (easily exhausted, failure to thrive, stomachache, repeated vomiting) and the PAI of his brother, this diagnosis was also established for him. Karyotype revealed a 46,XY male genotype and 21-hydroxylase deficiency were excluded. Per os prednisolone 5 mg/die and decosterone intramuscular injection 1 × 5 mg/week were commenced.

As with his older brother, his puberty was also delayed and the patient was treated with HCG 3000 IU/2 months from the age of 17 years. Due to this treatment testis became larger and secondary signs of male gender developed. A few years later because of the adherence problem he stopped the HCG, although he still stayed on glucocorticoid treatment (hydrocortisone 20 mg/day).

His registered heights were: at 7 years 125 cm (0.47 SD), 10 years: 131 cm (– 1.27 SD), 17 years: 155 cm (– 3.0 SD), 43 years: 200 cm (+ 3.78 SD) and weight 120 kg (BMI: 30 kg/m<sup>2</sup>).

At the age of 43 the diagnosis of PAI and HHG were proved by GnRH and ITT test. (Table 1). These tests revealed a low level of the gonadotropic hormones (peak LH: 0.3 IU/L, peak FSH: 1.4 IU/l), low cortisol (27.3 nmol/l), elevated ACTH (111.2 pg/ml) and low GH level (peak: 0.11 mIU/l). Similarly, to his brother the brain MR imaging revealed normal brain anatomy and a lack of any pituitary anomalies. In addition a molecular genetic examination showed the similar mutation detected in his brother.

From the age of 43 years his treatment included hydrocortisone 20 mg/day (8 mg/m<sup>2</sup>/day) hydrocortisone, mesterolone 50 mg/day, 0.1 mg fludrocortisone/day and vitamin D 30,000 IU/week. Moreover the androgen substitution (testosterone undecanoate 1000 mg) caused the same painful priapism as his brother, he also refused the replacement therapy of male sexual hormone.

## Molecular genetic analysis of DAX1 gene

Informed consent was obtained from the brothers and their mother for publication of their medical details. Molecular genetic examination of DAX1 was performed within the frame of the routine clinical genetic testing service at Clinical Genetics and Endocrinology Laboratory Semmelweis University following the institutional protocol. Genomic DNA was extracted from peripheral blood

leukocytes using the DNA isolation kit for mammalian blood (Boehringer Mannheim Corporation, Indianapolis, IN, USA) and DNA samples were frozen at  $-20^{\circ}\text{C}$  until they were analyzed. Mutation analysis of DAX1 gene was carried out using polymerase chain reaction (PCR) followed by direct bidirectional sequencing of the PCR-amplified DNA. The oligonucleotide primer pairs used for PCR amplification of exons 1 and 2 of DAX1 gene were as following:

1 AF: 5'-CGT GCG CGC TAG GTA TAA AT-3'  
 1 AR: 5'-GTT TGC TTT GCG CTC GTC-3'  
 1 BF: 5'-CAG GGC AGC ATC CTC TAC A-3'  
 1 BR: 5'-AAG CAG CAG CGG TAC AGA AG-3'  
 1 CF: 5'-CTT CTG TAC CGC TGC TGC TT-3'  
 1 CR: 5'-CCA CAG TCT CGA ACT GCA AG-3'  
 1 DF: 5'-CTT GCA GTT CGA GAC TGT GG-3'  
 1 DR: 5'-TTT TGT GAG CTG GGA AAA GC-3'  
 2 F: 5'-TTT TTC TCC CTC CAG ACG TG-3'  
 2 R: 5'-TCT TTT GCC CAC AGC TCT TT-3'

The PCR-amplified DNA was purified using a commercial kit (High pure PCR product, Roche Diagnostics, Mannheim, Germany). For direct sequencing of the whole coding region of DAX1 gene, Sanger sequencing was used. All sequences were compared to the NCBI reference sequence NG\_009814.1.

## Results

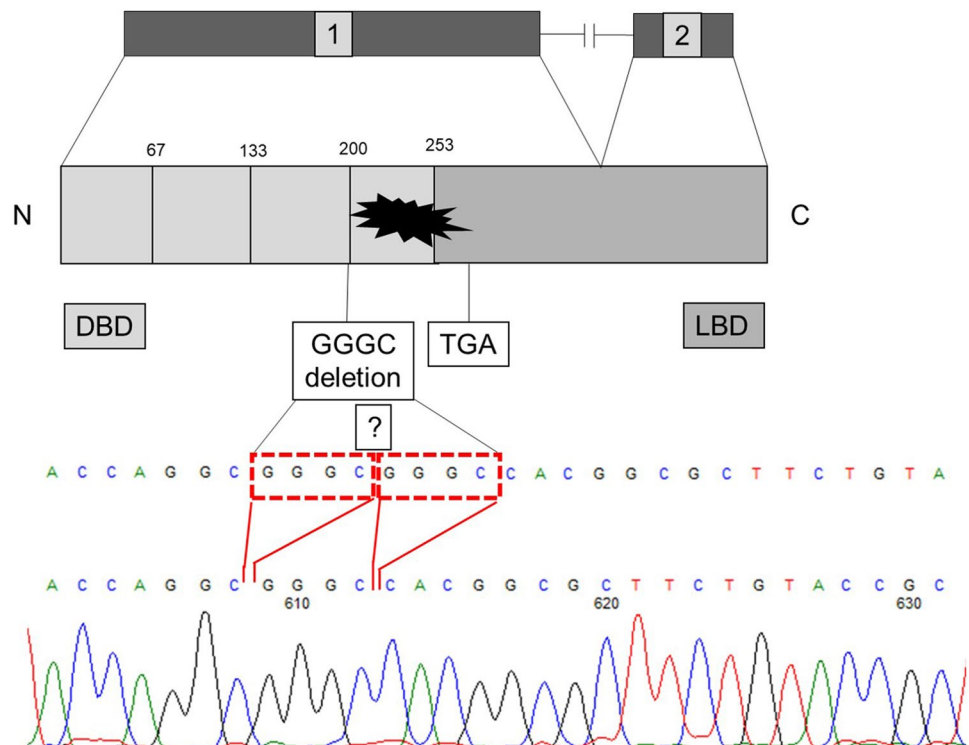
The clinical and endocrinological characteristics of the patients are summarized in Table 1. Direct sequencing of the NR0B1 (DAX1) gene revealed in both brothers a novel, four nucleotides deletion in the first exon (Fig. 1). The GGGC deletion is located in a sequence repetition GGGCGGGC, so deletion can be del.568-571c.GGGC or del.572-575c.GGGC. This four nucleotides containing deletion results in a frameshift and consequently the protein is terminated at an “ambush” Stop codon 788-790c. (Fig. 1). This mutation was also found in a heterozygous state in the patients' healthy mother.

This variant has not been reported in any public databases such as dbSNP (<http://www.ncbi.nlm.nih.gov/project/SNP>), ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) or ExAC (<http://exac.broadinstitute.org>).

## Discussion

DAX is a member of the nuclear hormone receptor family, although the homology is significant only in the putative carboxyl-terminal ligand binding domain (LBD). The DNA binding N terminal domain is substituted by an unusual novel domain that contains three and half repeats of a

**Fig. 1** A novel four nucleotides deletion del.586-571c.GGGC or 572-575c.GGGC of DAX1 gene in homozygous form



65–67 amino-acid motif containing two putative zinc fingers [18, 19].

In the current cases the deletion (c.568-571/572-575) locates just before the start of the ligand-binding domain (codon 196) (Fig. 1). Even the protein translation continues the four nucleotide deletion results in a frameshift and the structure of this part of the protein is not intact anymore. The “ambush” Stop codon at nucleotides 788–790 nucleotides cause deleterious damage of the ligand binding domain and this variant is considered pathogenic based on ACMG (American College of Medical Genetics) guideline.

DAX1 has a role in the maintenance of certain adult tissues, according to the Human Protein Atlas (<http://www.proteinatlas.org/ENSG00000169297-NR0B1/tissue>). It is expressed only in the adrenal gland, testis and ovaries, making impossible to test the functional consequences of the 4 nucleotides deletion on RNA level.

Several cases have been published when the LBD was abolished and the symptoms of hypoadrenia were presented only in childhood [18]. Nevertheless, even carrying the same mutation the age of the appearance of clinical symptoms can be very various [20]. One of the possible explanations of these facts is the differences in salvage mechanisms for persistence of fetal adrenal, which suggests that other factors, including epigenetics and protein modifications may be involved in the process [21, 22].

Affected boys with HHG fail to undergo puberty and require sexual hormone substitution. Repeated injections of hCG (human Choriogonadotropin) are generally successful in stimulating the testosterone production of the testis, but in practice the more accepted method is to use external testosterone replacement treatment to induce puberty, and the maintenance of sex-hormone replacement during adulthood [16]. In our patients the hCG treatment was successful, but after a two-year period the patients refused the intramuscular treatment and they stayed on the glucocorticoid, but without male sexual hormone replacements for two decades. Then in their adulthood induced testosterone undecanoate caused a priapism and due to this painful experience, they have also refused the androgen substitution therapy. According to patients they had never had an active sexual life. We suppose the long insufficient androgen replacement led to late fusion of the epiphysis consequently the patients reached their final height only at the end of their twenties and a serious osteoporosis developed in their forties.

According to Kletter and his colleagues in congenital adrenal hypoplasia with gonadotropin deficiency, the deficit in pituitary hormones is selective for gonadotropins (LH, FSH) and the other pituitary hormones' production remains intact (ACTH, GH, TSH and PRL) [14]. In our patients the diagnosis was proved at the ages of 51 and 43 years, as an ITT and GnRH test were implemented and beside the AHC and HHG, a low level of GH was established coincidentally.

The recommendation of Bornstein and colleagues does not suggest the measurement of growth hormone level in the case of AHC including the *DAX1* mutation suspect patients [23]. During the childhood of the brothers in Hungary the only accessible glucocorticoid product was the 5 mg prednisolone tablet. It is well known that the accurate dosing of this drug is difficult. Despite of this challenge, since introducing the prednisolone treatment the brothers had not had adrenal crisis and they did not show symptoms of overdose. From their young adult ages, they were treated with hydrocortisone. The Patient 1 required higher doses to be asymptomatic, but the Patient 2 was treated in substitution dose of hydrocortisone supplemented both of them with mineralocorticoid. Despite of the disadvantageous prednisolone treatment on the bone maturation the final adult heights of the brothers were + 1.85 and + 3.78 SD.

Calliari and colleagues published a case about a boy with *DAX1* mutation and below average adult height. In this case the growth hormone level was not measured and the induction of puberty was started at 12 years of age due to psychological aspects [24]. In two patients published by Rojek the growth hormone deficiency was diagnosed at the age of 13 and 18 years respectively. In the second case, due to the retarded bone age, the latterly induced sexual hormone replacement and growth hormone therapy in the patient still showed growth at 23 years of age [25].

In cases of glucocorticoid treatment, the GH deficiency should be interpreted as a consequence of the excess of glucocorticoids substitution [25]. Although in our cases the suppressed ACTH level has not been proved, we could not exclude the harmful effect of glucocorticoids. However the fact, that the patients had still significant growth after 16 years of age (35 and 45 cm) does not confirm the overdose of glucocorticoid administered during this period.

In our patients, beside the low normal range measured IGF level, significantly low GH concentration were detected during the ITT, which can be explained by the fact that they were not on adequate male sexual hormone therapy before and during the test. Nevertheless, we suppose the main reason of the elevated BMI is their sedentary lifestyle which may be related to the mesterolone therapy instead of a proper male sexual hormone replacement.

In conclusion, in our two male patients with AHC and HHG, caused by a novel *DAX1* mutation despite of the long prednisolone treatment in their childhood they had reached a tall adult height. While the brothers had a significant growth during their adulthood, we assume the lack of the sexual hormone substitution was the main reason of their above average height as well as their serious osteoporosis.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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